The Optokinetic Response of Fishes to Different Levels of Turbidity

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Abstract:

Many fish need light to school, reproduce, and forage. Without enough light penetrating into an aquatic system fish may not be able to accomplish these tasks, thus compromising their ability to persist. Turbidity, or suspended particles in the water, is a serious global problem due to increased run-off from urban and agricultural activities. High turbidity has the potential to reduce light to a point where fish are unable to detect the visual environment. The first objective to this project was to develop an optomotor response apparatus for testing the visual abilities of fish under increasing turbidity. Due to the optokinetic response, fish will swim with a rotating black and white grating until the turbidity reaches a peak where they can no longer sense the white striations, at which point the fish can no longer see the gradient and stops swimming (i.e. detection threshold). Under the parameters of 8 rotations per minute, striations 35 mm wide, and broad-spectrum lighting 15 individual fish showed responses to the rotating gradients. For the second objective, I used the optomotor apparatus to test the detection threshold under increasing turbidity for *Pseudocrenilbrus multicolor victoria*, a widespread East African cichlid fish that experiences extremes of human-induced turbidity. All fish that exhibited the optokinetic response (n = 15) were tested for a turbidity threshold. Males showed a significantly higher turbidity threshold (mean ± s.e. = 61.94 ± 3.03) level compared to females (mean ± s.e. = 52.64 ± 2.92). This research is beneficial because it can be applied to many different fish species experiencing increases in turbidity above natural levels and may contribute to our understanding of the mechanisms of population declines associated with increased turbidity.

Introduction:

Throughout the world, aquatic biodiversity is being lost in large numbers due to many anthropogenic effects and the impact on humans is starting to be apparent. In Lake Victoria, East Africa,human-introduced species, such as the Nile Perch (*Lates niloticus*), have decimated endemic species of fish (Ogutu-Ohwayo 1990). Overfishing of inland waters has also reduced biodiversity in freshwater systems and humans are therefore suffering because of the lack of resources available (Allan et al. 2005). One major cause of biodiversity loss in freshwaters is due to high levels of human-induced turbidity in natural water systems (Dudgeon et al. 2005). Turbidity is the cloudiness or haziness of water caused by suspended particles (Utne-Palm, 2002). This reduces water clarity and can be detrimental to water quality. For example, increased turbidity can lead to decreased primary production because of decreased light penetration, which in certain food webs can cascade up through trophic levels causing population problems for prey species as well (Henley et al. 2005). Increased turbidity is a global problem resulting from agricultural and urban runoff (Dudgeon et al. 2005). Increased turbidity occurs both naturally and by human activities. When arid areas or landscapes with altered land use from deforestation are impacted with heavy rains, flash floods can occur and large sediment loads can be displaced and carried through waterways, drastically increasing turbidity. Increased turbidity can also be caused by agricultural and urban runoff in the forms of algal turbidity and large sediment displacements (Fichez et al. 1992). Regardless of the source, the impact of increased turbidity on biodiversity is being detected frequently and is therefore in need of a better management strategy.

Increased turbidity that is higher than natural levels has been shown to be a key factor in the loss of freshwater biodiversity (Dudgeon et al. 2005). In impacted systems, elevated turbidity can reach such high levels that it may restrict a fish's ability to visually sense the surrounding

environment (Kroger 2003). Freshwater habitats with different underwater light environments influence how fish detect each other, dependent on which wavelengths of light are most abundant (Fuller 2002). Suspended particles in the water scatter light and absorb different wavelengths making certain, thus changing the spectrum of light (i.e. color of light underwater). Many fish need light in order to forage, avoid predators, school, and reproduce; without light many fish may therefore be limited to only a certain number of visually-mediated actions (Hogan & Laskowski 2013). When fish are restricted to limited amounts of light they may experience population declines due to the lack of functions being able to be performed (Pita et al. 2015). For example, in Lake Victoria there were recently an estimated 500 unique species of cichlid fish (Seehausen et al. 1997). Within the past decade Lake Victoria has rapidly eutrophied and water clarity has reduced greatly. Many cichlid fish species use certain color cues to locate a conspecific mate and to avoid mating with heterospecific mates. With the increased turbidity in Lake Victoria, cichlids were less able to identify color species-specific color cues leading to homogenization of the species flock. The lack of broad-spectrum lighting due to high turbidity disrupted reproductive isolation between cichlid species, leading to decreased biodiversity (Seehausen et al. 1997). With the problem of excessive turbidity increasing globally it is critical to know the visual detection thresholds of fish experiencing altered underwater light environments in order to conserve fish diversity. In very turbid waters fish can become limited in their visual sensitivity due to the lack of clarity in the water (Mueller et al. 2010). We therefore expect that for each individual or species there is a turbidity level at which the fish's detection threshold is reached, therefore hindering behaviorally mediated activities.

Research has shown that we can determine a fish's detection threshold by taking advantage of the optokinetic response (Maan et al. 2006). The optokinetic response is an innate

physiological feature in which fish respond instinctively to a moving object (Sperry 1950). An example of the optokinetic response would be t that an aquarium fish will follow the motion of your finger around the glass as you move it in a figure eight fashion. Optomotor devices have been used to detect the optokinetic response of fishes in a controlled setting. Maan et al. (2006) used optomotor tests to assess the sensory drive hypothesis in cichlids: testing if different photic environments contribute to the evolution of reproductively isolated species. The experiment tested if the cichlids could detect light under alternating intensities and colors, representing different turbid environments.

In this study we investigated two objectives. First, we wanted to evaluate if the optokinetic response could be used to understand detection thresholds under turbid conditions. Many studies have used the optokinetic response to test detection thresholds using different light intensities or colors of light (Sperry 1950), however no one to our knowledge has directly manipulated turbidity levels. In order to complete this task it was necessary to develop a functioning optomotor apparatus to test the visual abilities of fish under increasing levels of turbidity. The second objective of this study was to test the detection threshold under increasing turbidity of *Pseudocrenilabrus multicolor victoriae*, a widespread East African cichlid that experiences extremes of human-induced turbidity. The males and females of this sexually dimorphic fish (Fig. 1 and 2) play very different roles in reproduction and so we expect that they might have different detection thresholds.

Methods:

Optomotor apparatus design

Multiple optomotor device designs have been used to detect the optokinetic response in fish and each have similarities and differences (Maan et al. 2006). Maan et al. (2006) used different wavelengths of light throughout different trials of the optokinetic response while in this experiment all spectrums of light were used at all times. The design used in this experiment is a unique set-up, but also has the major components of other optomotor apparatuses (Fig. 3). In this design, there was a glass cylindrical tank that had a diameter of 20.32 centimeters and was purposed to house the sample fish during trials. The tank was kept stable by the use of high tensile strength clamps and para-cord draped over a utility rack. This design was strong enough to hold the tank for hours while still being weighted with water. Underneath this suspended tank was a standard record player. Above the tank we hung a broad-spectrum light that was used to represent natural sunlight. Adjacent to the light was a mounted video camera (Canon Vixia HF R600 High Definition Camcorder) to capture video of the optokinetic response of the fish being tested. Placed around the tank and anchored to the record player was a black and white striped gradient that acted as the trigger mechanism for the optokinetic response. The striations used for this experiment were 35 mm wide and both black and white striations were the same width and alternated one after the other (Maan et al. 2006).

Study Species

The study species used in this experiment was the cichlid species *Pseudocrenilabrus multicolor victoriae* (Fig. 1 and 2). This species of cichlid is endemic to the Lake Victoria region of Eastern

Africa and is found in different habitats throughout the region (Chapman et al. 2002). Some habitats these fish are found in are stagnant and clear swamp systems. *P. multicolor* can also be found in moving water systems that are very turbid (Chapman et al. 1996). The male fish of this species are normally a vibrant yellow color with prominent blue lips and other blue and red markings on the anal fins (Fig. 1). They females are gray-brown with tinges of blue on the scales and fins (Fig. 2). Males use their vibrant colors to display for a potential mate, or they use them to defend their territories against other males who could be potential threats (Gray et al. 2012). *P. multicolor* uses vision as a primary sense in reproduction. By limiting vision due to turbidity this species may suffer due to the fact that they may not be able to detect each other in turbid versus clear water. The specific fish used for this experiment were either first generation *P. multicolor* collected in Uganda or an F1 generation of a mixed population from parents originating from swamp or river habitats.

Visual Detection Trials

Trials were executed after the design of the optomotor device was completed and we had established that the fish would respond to a grating of 35 mm size and speed of 8 rotations per minute (Maan et al. 2006). Different acclimation periods were conducted in the preliminary trials to determine which time period guaranteed the optokinetic response (acclimation times = 10 minutes and 30 minutes). Smaller gradient sizes were also tried to determine which width of gradients generated the most apparent response. Each fish was placed in 800 ml of water in the cylindrical tank and let to sit for a 15-minute acclimation period. Once the fish was settled the gradient was activated to spin around the tank at a speed of 8 rotations per minute (Maan et al. 2006). The fish demonstrated the optokinetic response for 2 minutes in clear water before any

turbidity solution was added. The turbidity solution was created by mixing 5g of bentonite clay with 50ml of treated water (NovAqua). The turbidity solution was added in increments of 0.08 mL, which increased turbidity in the tank approximately 2 NTUs for every addition of turbidity solution (Fig. 4). The water within the tank was homogenized with the turbidity solution by recirculating the water through a 30mL pipette after each addition. The turbidity solution was incrementally added until the fish within the tank stopped swimming in the clockwise rotation and responding to the black and white gradient. A total of 15 fish were tested for turbidity thresholds and each fish was run through two trials to have a standardized result, and both of those measurements were used to calculate a mean NTU threshold level for each fish.

Results:

The overall design of the optomotor apparatus was successful in generating the optokinetic response from *P. multicolor.* With gradients 35 mm wide and spinning at a speed of 8 rotations per minute the sample fish responded in clear and turbid water. In the preliminary trials 5 fish were used to generate an optokinetic response in only clear water to solidify the capabilities of the device and the parameters being used.

The optokinetic response was generated in 15 different fish consisting of 8 females and 7 males (Table 1 and 2). The mean (± S.E.) detection threshold for all fish was 57.02 (± 2.4) NTU. The average threshold level for males was 61.94 (± 3.0) NTU (Fig. 5). The average threshold level for females was 52.64 (± 2.9) NTU (Fig. 5). A two-tailed unpaired t-test was conducted between male and female turbidity threshold. The t-test was done with a 95% confidence interval

and generated a t-value = 2.2025 and a P-value =0.046, indicating a statistically significant difference between male and female turbidity thresholds.

Discussion:

A successful optomotor apparatus was designed through the creative and repurposed use of materials. The novel idea of using a record player to rotate the striped gradient worked very well. The optomotor device was able to successfully generate an optokinetic response in the available fish specimens; *P. multicolor* had a physiological response to the optomotor apparatus. The turbidity was successfully increased throughout trials to show how fish react in different turbid settings. One notable observation from the experiment was that males showed higher turbidity threshold levels than females (Fig. 3). One conclusion that can be made from this is that males are therefore able to visually sense females before females detect males under turbid conditions; potentially giving males the ability to court females with a colorful behavioral display before other males compete for the same female mate. The levels of turbidity produced simulated natural settings from human induced changes in the environment (Kasangaki et al. 2008).

The idea of using an optomotor device for testing visual acuity in turbid waters is just one way to test responses to turbidity. Other designs have focused on the concept of reaction distance and have used long rectangular tanks instead of cylindrical ones. The optomotor response has been used in many different fish and mammals (Sperry 1950), but this experiment was the first time that fish have been tested with the optokinetic response to analyze their response to turbidity. In another experiment, Gregory & Northcote (1993) used reaction distance to

determine when juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) would react to a prey source and observed that reaction distance decreased as turbidity increased. The advantage of using the optokinetic response to test turbidity thresholds compared to reaction distance is that turbidity can be changed during the actual trial using an optomotor apparatus. By being able to increase turbidity during a trial you can increase NTUs in smaller increments and therefore get a more accurate determination of the turbidity threshold. Turbidity scatters light and makes objects harder to detect (Utne-Palm 2002). By using a broad-spectrum light in this experiment we were able to simulate a natural, daylight setting and proved that with increased turbidity organisms that rely on vision become significantly visually impaired at certain levels of turbidity. Using the optomotor apparatus will allow other species of fish to be analyzed in a controlled environment and determine their turbidity thresholds. High levels of human induced turbidity are certainly a global issue. Different fish species may be encountering population dynamic problems and the issue could be excessive turbidity (Henley et al. 2000). By testing different species of fish using the optokinetic response to turbidity, management plans can be made and enforced to compensate for the overwhelming turbidity in areas that fish are facing severe problems.

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Fig 1. Male *P. multicolor*

Fig 2. Female *P. mulitcolor*

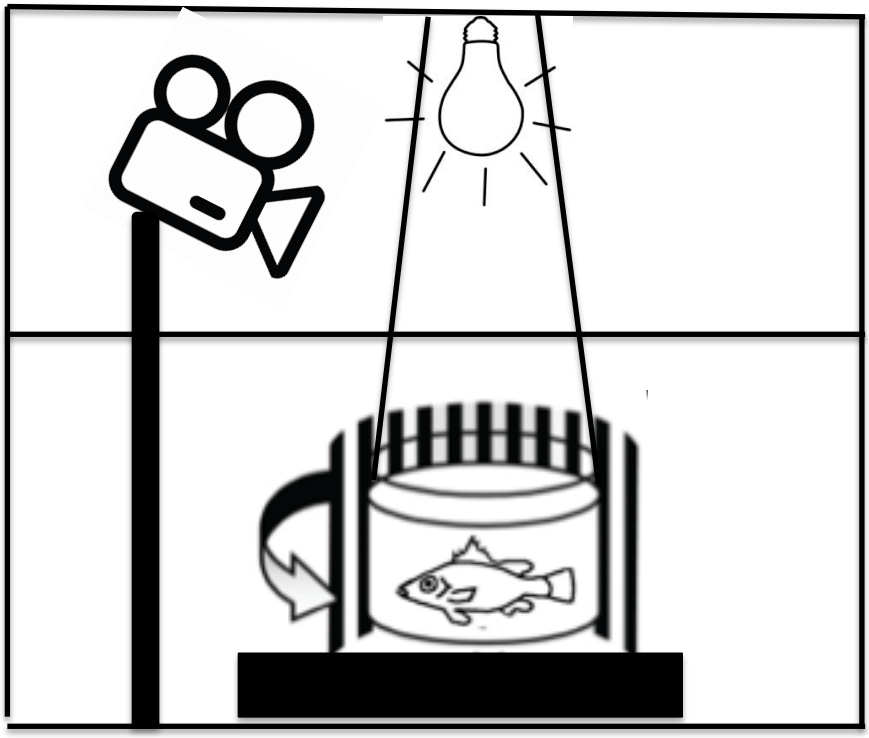

Figure 3. Modified design of the optomotor apparatus from Maan et al (2006). The cylindrical tank is suspended above a standard record player using para-cord tied to clamps that are attached to the edge of the tank. This design holds the tank level above the record player. On top of the record player sits the gradient that spins around the suspended tank. Hanging directly above the record player is a broad-spectrum light. Adjacent the light is a video camera to capture the behavior of the fish.

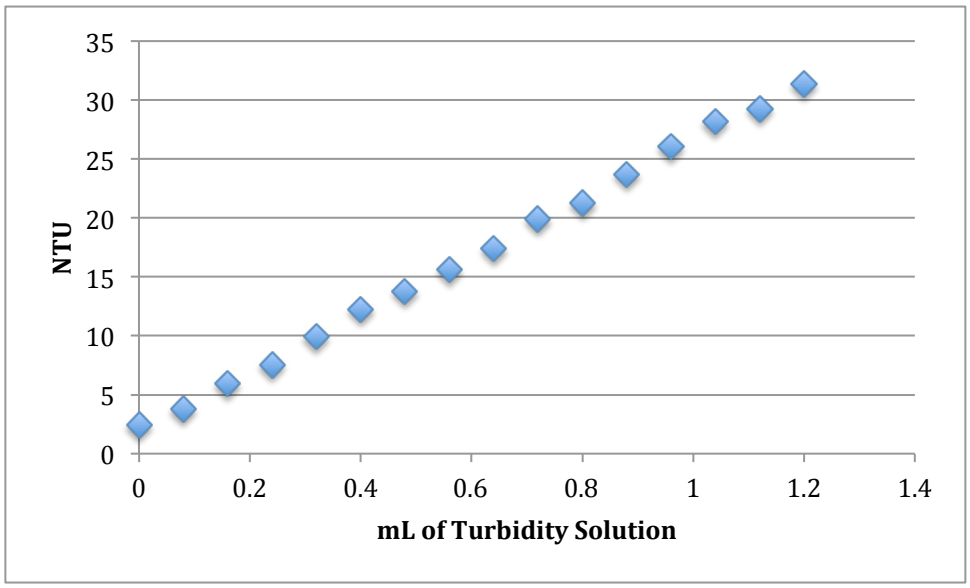

Figure 4. Standardized additions of turbidity solution and their change of the NTU to the existing water. A bentonite clay solution was made to increase turbidity. The clay solution was added with a standard 2.5mL pipette. Every addition of the solution was 0.08mL and increased the turbidity approximately 2 NTUs.

Table 1. Female fish specimens and their average visual thresholds

Female Fish ID	NTU Threshold
2	52.78
3	44.73
4	44.17
5	53.86
7	48.95
9	59.59
10	68.84
14	48.24

Table 2. Male fish specimens and their average visual thresholds

Male Fish ID	NTU Threshold
1	60.81
6	69.17
8	65.53
11	48.17
12	69.55
13	65.94
15	54.45

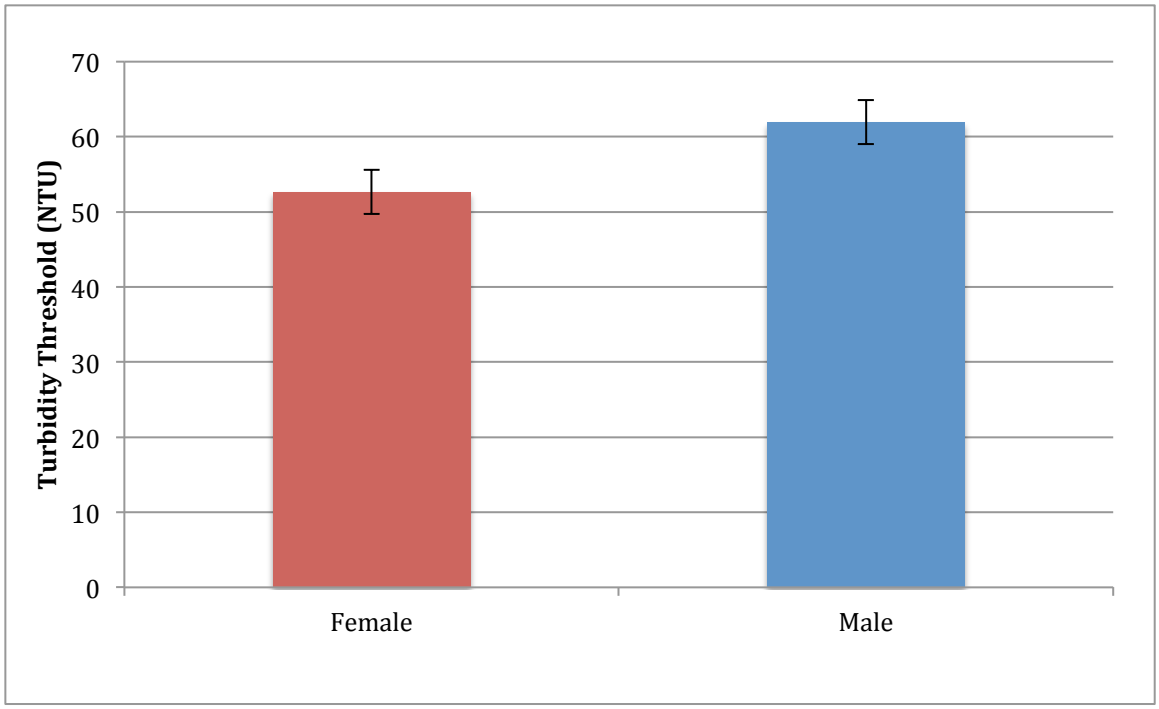

Figure 5. The mean (± s.e.) visual acuity thresholds for male and female *P. multicolor* under incrementally increased turbidity (NTU)